sample solution; and
m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 20 milligrams of sulbactam per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the sulbactam working standard.

[52 FR 42290, Nov. 4, 1987; 52 FR 45281, Nov. 25, 1987, as amended at 54 FR 47205, Nov. 13, 1989; 55 FR 11585, Mar. 29, 1990]

§455.85 Vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(v) It contains not more than 15 percent of factor A.

(vi) It gives a positive identity test for vancomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, factor A content, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(5) *Identity and factor A content.* Proceed as directed in §455.85a(b)(7).

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§455.85a Sterile vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5 percent.

(vi) Its pH in an aqueous solution containing 50 milligrams per milliliter

is not less than 2.5 and not more than 4.5.

(vii) Its heavy metals content is not more than 30 parts per million.

(viii) It contains not more than 15 percent of factor A.

(ix) It gives a positive identity test for vancomycin.

(2) *Packaging.* In addition to the requirements of § 432.1 of this chapter, if it is packaged for dispensing, the vancomycin content of each immediate container is 500 milligrams of vancomycin.

(3) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, heavy metals, factor A content, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 12 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to give a stock solution of 1 milligram per milliliter. Further dilute an aliquot of the stock solution with solution 4 to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use sterile distilled water in lieu of diluting fluid A.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of vancomycin per milliliter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(7) *Identity and factor A content*—(i) *Preparation of the chromatogram*—(*a*) *Equipment.* (*1*) Chromatographic paper (Whatman No. 1 untreated filter paper).

(*2*) Equipment for descending paper chromatography (Mitchell tank).

(*b*) *Preparations of solutions*—(*1*) *Factor A.* Prepare a solution in distilled water to contain 1.33 milligrams of factor A per milliliter and further dilute with distilled water to prepare solutions containing 0.1 and 0.2 milligram of factor A per milliliter.

(*2*) *Vancomycin working standard solution.* Prepare a solution in distilled water to contain 1.33 milligrams of vancomycin per milliliter.

(*3*) *Known mixture of factor A and vancomycin.* Prepare a solution in distilled water to contain 0.2 milligram of factor A and 1.13 milligrams of vancomycin (estimated) per milliliter.

(*4*) *Sample.* Prepare two solutions of the sample in distilled water, each to contain 1.33 milligrams of vancomycin (estimated) per milliliter.

(5) *Solvent mixture.* Mix 300 milliliters of butyl alcohol, 150 milliliters of pyridine, and 200 milliliters of water in a large separatory funnel and shake well for 3 minutes. Let stand at room temperature. There should be no separation of layers.

(*c*) *Procedure.* Saturate the atmosphere in the tank with vapors of the

solvent mixture by placing 10 milliliters of the mixture in a trough in the bottom of the tank and closing tightly for 15 minutes. Prepare a sheet of chromatographic paper (8 inches x 8 inches) by carefully drawing a line of origin with a pencil 2 inches from one of the edges. Fold the paper along a straight line 1½ inches from the same edge of the paper. Starting 1 inch from the left-hand edge, establish points at 1-inch intervals along the line of origin on which to apply the solutions. Using a micropipette, apply the factor A solutions, the vancomycin solution, the known mixture solution, and the sample solutions by placing 5 microliters of each on separate spots. Properly identify the locations of the spots but avoid unnecessary handling of the paper. Allow the spots to dry spontaneously. Suspend the paper in the chamber so that the edge nearest the fold can be conveniently immersed in the solvent mixture contained in the top trough. Immerse the paper across its entire width to a depth sufficient to assure contact with the solvent mixture during the entire development time. Close the chamber tightly and allow the chromatograph to develop at room temperature for 6½ to 7 hours. Remove the paper and allow it to dry completely.

(ii) *Development by bioautograph*—(*a*) *Preparation of test organism* (*spore suspension*). The test organism is *Bacillus subtilis* (ATCC 6633),[1] test organism H, prepared as described in § 436.103 of this chapter, using the method described in paragraph (b)(2) of that section.

(*b*) *Preparation of plates*—(*1*) *Baselayer.* Add 42 milliliters of medium 2 described in § 436.102(b)(2) of this chapter to each Petri dish (25 millimeters x 150 millimeters) and allow to harden on a flat, level surface. To prevent condensation of excess moisture, raise the tops slightly while the agar hardens.

(*2*) *Seed layer.* Melt nutrient agar medium 2 described in § 436.102(b)(2) of this chapter. Accurately measure a sufficient quantity of the melted agar, cool to 48° C., and add the appropriate quantity of the spore suspension prepared as described in paragraph (b)(7)(ii)(*a*) of this section. Swirl the flask of inoculated agar to obtain a homogeneous suspension. Add 8 milliliters of this inoculated agar to each plate, spread evenly, and allow to harden on a flat, level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the entire surface of the plates.

(*c*) *Assay.* For each spot on the paper described in paragraph (b)(7)(i)(*c*) of this section, cut a strip 1.5 centimeters by approximately 14 centimeters with the center of each strip centered about the line of descent of the spot. Place all strips on plates with the aid of forceps within as short a period of time as possible. Use maximum spacing between strips. Insure complete contact so that the entire strip becomes uniformly moistened. Allow to stand for 30 minutes. Remove the strips and identify each strip location on the Petri dish. Incubate the plates for 16–18 hours at 37° C. Any zone of inhibition corresponding to factor A in the sample must not be greater than that of the 0.2 milligram-per-milliliter factor A standard. Also, the two areas of inhibition for the sample due to the presence of factor A and vancomycin must compare to the corresponding two areas of inhibition of the known mixture in their respective distances from their origins.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.86 Vancomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin is a tricyclic glycopeptide. It is a free flowing white to off-white colored powder. It is so purified and dried that:

(i) It contains not less than 925 micrograms of vancomycin per milligram, calculated on the anhydrous basis.

(ii) It contains not less than 92 percent vancomycin factor B and not more than 3 percent of any individual vancomycin related factor.

(iii) Its moisture content is not more than 20 percent.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.